Claims

1. A pharmaceutical composition comprising

a pharmaceutically acceptable carrier, wherein said composition comprises:

a) a therapeutically effective amount of an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4, or a homologous peptide thereof and/or a vector comprising a nucleic acid molecule that comprises the nucleotide sequence that encodes an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4, or a homologous peptide thereof.;

b) a therapeutically effective amount of a non-ECM binding fragment of Ang-1 protein that comprises a modification in an ECM-binding domain of Ang-1, wherein said modification reduces the binding of Ang-1 to an extracellular matrix (ECM) and/or a vector comprising a nucleic acid molecule that comprises the nucleotide sequence that encodes a non-ECM binding fragment of Ang-1 protein that comprises a modification in an ECM-binding domain of Ang-1, wherein said modification reduces the binding of Ang-1 to the ECM; or

c) a therapeutically effective amount of a proteolytic resistant fragment of Ang-1 protein that comprises a modification in a proteolytic domain of Ang-1, wherein said modification inhibits the proteolysis of Ang-1 and/or a vector comprising a nucleic acid molecule that comprises the nucleotide sequence that encodes a proteolytic resistant fragment of Ang-1 protein that comprises a modification in a proteolytic domain of Ang-1, wherein said modification inhibits the proteolysis of Ang-1.

2. The pharmaceutical composition of claim 1 comprising a therapeutically effective amount of an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4.

3. The pharmaceutical composition of claim 1 comprising a therapeutically effective amount of a non-ECM binding fragment of Ang-1 protein that comprises a modification in an ECM-binding domain of Ang-1, wherein said modification reduces the binding of Ang-1 to an extracellular matrix (ECM).

4. The pharmaceutical composition of claim 3 wherein said modification is a substitution, insertion, or deletion.

5. The pharmaceutical composition of claim 3 wherein said non-ECM binding fragment of Ang-1 protein comprises SEQ ID NOs:5, 6, 7, 8, 9, and/or 10

6. The pharmaceutical composition of claim 1 comprising a therapeutically effective amount of a proteolytic resistant fragment of Ang-1 protein that comprises a modification in a proteolytic domain of Ang-1, wherein said modification inhibits the proteolysis of Ang-1.

7. The pharmaceutical composition of claim 6 wherein said proteolytic resistant fragment of Ang-1 protein comprises a modification in a sequence comprising SEQ ID NOs: 1 and/or 2.

8. The pharmaceutical composition of claim 6 wherein said modification is a substitution, insertion or deletion.

9. The pharmaceutical composition of claim 6 wherein said proteolytic resistant fragment of Ang-1 comprises SEQ ID NOs:5, 6, 9, and/or 10.

10. The pharmaceutical composition of claim 1 comprising a vector comprising a nucleic acid molecule that comprises the nucleotide sequence that encodes an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4.

11. The pharmaceutical composition of claim 1 comprising a vector comprising a nucleic acid molecule that comprises the nucleotide sequence that encodes a non-ECM binding fragment of Ang-1 protein that comprises a modification in an ECM-binding domain of Ang-1, wherein said modification reduces the binding of Ang-1 to an extracellular matrix (ECM).

12. The pharmaceutical composition of claim 1 comprising a vector comprising a nucleic acid molecule that comprises the nucleotide sequence that encodes a proteolytic resistant fragment of Ang-1 protein that comprises a modification in a proteolytic domain of Ang-1, wherein said modification inhibits the proteolysis of Ang-1.

13. The pharmaceutical composition of claim 10 wherein said vector is a viral vector.

14. The pharmaceutical composition of claim 11 wherein said vector is a viral vector.

15. The pharmaceutical composition of claim 12 wherein said vector is a viral vector.

16. The pharmaceutical composition of claim 10 wherein said vector is a DNA plasmid.

17. The pharmaceutical composition of claim 11 wherein said vector is a DNA plasmid.

18. The pharmaceutical composition of claim 12 wherein said vector is a DNA plasmid.

19. A pharmaceutical composition comprising

a pharmaceutically acceptable carrier and

a therapeutically effective amount of a mutant Ang-1 which retain their angiogenesis promoting activity but which have reduced or inactive ECM binding or a homologous peptide thereof or mutant versions of Ang-1 or a homologous peptide thereof and/or a vector comprising a nucleic acid molecule that comprises the nucleotide sequence that encodes a mutant Ang-1 which retain their angiogenesis promoting activity but which have reduced or inactive ECM binding or a homologous peptide thereof or mutant versions of Ang-1.

20. The pharmaceutical composition of claim 19 wherein the mutant Ang-1 which retain their angiogenesis promoting activity but which have reduced or inactive ECM binding or a homologous peptide thereof or mutant versions of Ang-1 is missing an ECM binding motif or has a substitution(s), deletion(s) or insertions within an ECM binding motif.

21. The pharmaceutical composition of claim 20 wherein said mutant Ang-1 comprises SEQ ID NOs:5,6, 7, 8, 9, and/or 10.

22. The pharmaceutical composition of claim 19 comprising a therapeutically effective amount of a mutant Ang-1 which retain their angiogenesis promoting activity but which have reduced or inactive ECM binding or a homologous peptide thereof or mutant versions of Ang-1.

23. The pharmaceutical composition of claim 19 comprising a vector comprising a nucleic acid molecule that comprises the nucleotide sequence that encodes a mutant Ang-1 which retain their angiogenesis promoting activity but which have reduced or inactive ECM binding or a homologous peptide thereof or mutant versions of Ang-1.

24. The pharmaceutical composition of claim 23 comprising wherein said vector is a viral vector.

25. The pharmaceutical composition of claim 23 comprising wherein said vector is a DNA plasmid.

26. A pharmaceutical composition comprising

a pharmaceutically acceptable carrier and

a therapeutically effective amount of a mutant Ang-1 which retain their angiogenesis promoting activity but which is not cleaved into a antagonist fragment or a homologous peptide thereof or a homologous peptide thereof or and/or a vector comprising a nucleic acid molecule that comprises the nucleotide sequence that a mutant Ang-1 which retain their angiogenesis promoting activity but which has is not cleaved into a antagonist fragment or a homologous peptide thereof.

27. The pharmaceutical composition of claim 26 comprising a therapeutically effective amount of a mutant Ang-1 which retain their angiogenesis promoting activity but which is not cleaved into a antagonist fragment or a homologous peptide thereof.

28. The pharmaceutical composition of claim 26 wherein said mutant Ang-1 comprises SEQ ID NO: 5, 6, 9, or 10.

29. The pharmaceutical composition of claim 26 comprising a vector comprising a nucleic acid molecule that comprises the nucleotide sequence that encodes a mutant Ang-1 which retain their angiogenesis promoting activity but which has is not cleaved into a antagonist fragment or a homologous peptide thereof.

30. The pharmaceutical composition of claim 29 wherein said nucleic acid molecule is SEQ ID NO: 23, 24, 27, and/or 28.

31. The pharmaceutical composition of claim 27 comprising wherein said vector is a viral vector.

32. The pharmaceutical composition of claim 27 comprising wherein said vector is a DNA plasmid.

33. A method of treating an individual suspected of having coronary artery disease, vascular disease or a condition involving ischemia comprising the step of administering to said individual a pharmaceutical composition according to any of claims 1-32.

34. A method of promoting angiogenesis, endothelial survival and maintaining vascular integrity in an individual comprising the step of administering to said individual a pharmaceutical composition according to any of claims 1-32.

35. A method of treating an individual suspected of having a disease related to lack of blood vessels to effectively promote angiogenesis in the patients with the diseases related to lack of blood vessels such as ischemia in hearts and limbs comprising the step of administering to said individual a pharmaceutical composition according to any of claims 1-32.

36. A method to reduce stroke, heart attack, blood vessel blockage, hemorrhage, artherosclerosis risk by maintain the health and integrity of blood vessels (by reduce the loss the endothelial monolayer integrity and attachment of blood cells on vessel walls) comprising the step of administering to said individual a pharmaceutical composition according to any of claims 1-32.

37. A method to assist the recovery of the patients who had stroke and the angioplasty procedure by promoting the growth/survival of endothelial cells and establish endothelial monolayer and inhibit excessive inflammation, hemorrhage, and proliferation of vascular smooth muscle. comprising the step of administering to said individual a pharmaceutical composition according to any of claims 1-32.

38. A method to treat patients with restenosis by inhibiting re-closure of blood vessel after inserting stents into blood vessels. comprising the step of administering to said individual a pharmaceutical composition according to any of claims 1-32.

39. A method to make stable and functional artificial blood vessels comprising the step of using a composition according to any of claims 1-32.

40. A method of identifying compounds that modulates binding of Ang-1 to ECM comprising performing a test assay that comprises the steps of :

a) contacting a protein that comprises at least an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4 with ECM material in the presence of a test compound;

b) measuring the level of binding of said protein that comprises at least an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4 with said ECM; and

c) comparing said level with the level of binding of protein that comprises at least an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4 with ECM material in the absence of said test compound;

wherein

when the level of binding of said protein that comprises at least an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4 with said ECM in the presence of said test compound is less than the level of binding of said protein that comprises at least an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4 with said ECM in the absence of said test compound results indicate that the test compound modulates binding of Ang-1 to ECM by inhibiting said binding and

when the level of binding of said protein that comprises at least an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4 with said ECM in the presence of said test compound is more than the level of binding of said protein that comprises at least an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4 with said ECM in the absence of said test compound results indicate that the test compound modulates binding of Ang-1 to ECM by enhancing said binding.

41. The method of claim 40 wherein the protein is Ang-1 protein.

42. The method of claim 40 wherein the protein is a fragment of Ang-1 protein.

43. The method of claim 42 wherein said fragment comprises SEQ ID NOs:1, 2, 3, and/or 4.

44. The method of claim 40 wherein the protein comprises a detectable label.

45. The method of claim 44 wherein the level of binding is measured by measuring the amount of detectable label present on said ECM after removing unbound protein from said ECM.

46. The method of claim 40 wherein the ECM material is produced by culturing cells on a substrate for a sufficient time for said cells to produce ECM material on said substrate and removing said cells from said substrate without removing said ECM material.

47. The method of claim 46 wherein said cells are Lewis lung carcinoma cells or TA3 murine mammary carcinoma cells.

48. The method of claim 40 wherein said method comprising multiple test assays which are identical except that the amount of test compound used differs.

49. The method of claim 40 the level of binding of protein that comprises at least an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4 with ECM material in the absence of said test compound is determined by performing a control assay wherein said control assay comprises the steps of

a) contacting a protein that comprises at least an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4with ECM material in the absence of a test compound;

b) measuring the level of binding of said protein that comprises at least an ECM-binding fragment of Ang-1 protein that comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4 with said ECM.

50. The method of claim 40 wherein said compound is a peptide.

51. The method of claim 40 wherein said peptide is a fragment of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and/or SEQ ID NO:4.

52. The method of claim 51 wherein said fragment is at least 5 amino acid residues in length.

53. A pharmaceutical composition comprising

a) a pharmaceutically acceptable carrier and

b) a therapeutically effective amount of an Ang-1 fragment with antagonist activity and/or a vector comprising a nucleic acid molecule that comprises the nucleotide coding sequence of an Ang-1 fragment with antagonist activity.

54. The pharmaceutical composition of claim 53 further comprising Ang-2 protein and/or a vector comprising a nucleic acid molecule that comprises the nucleotide coding sequence of Ang-2.

55. The pharmaceutical composition of claim 53 comprising a therapeutically effective amount of an Ang-1 fragment with antagonist activity.

56. The pharmaceutical composition of claim 53 wherein said Ang-1 fragment with antagonist activity comprises an amino acid sequence comprising SEQ ID NO:11 and/or SEQ ID NO:12 and/or a vector comprising a nucleic acid molecule that comprises the nucleotide sequence encoding for SEQ ID NO:29 and/or SEQ ID NO:30.

57. The pharmaceutical composition of claim 53 comprising a vector comprising a nucleic acid molecule that comprises the nucleotide coding sequence of an Ang-1 fragment with antagonist activity.

58. The pharmaceutical composition of claim 57 wherein said nucleotide coding sequence comprises SEQ ID NO:29 and/or SEQ ID NO:30.

59. The pharmaceutical composition of claim 57 comprising wherein said vector is a viral vector.

60. The pharmaceutical composition of claim 57 comprising wherein said vector is a DNA plasmid.

61. A method of treating an individual suspected of having cancer comprising the step of administering to said individual a pharmaceutical composition according to any of claims 53-60.

62. The method of claim 61 wherein the composition is administered in conjunction with removal or elimination of a tumor.

63. A method of preventing diabetes and/or arthritis in an individual suspected of being at risk of developing diabetes or arthritis comprising the step of administering to said individual a pharmaceutical composition according to any of claims 53-60.

64. A fusion protein comprising an ECM binding motif SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and/or SEQ ID NO:4 and a biologically active non-Angl protein.

65. A fusion protein comprising a non-ECM binding fragment of ang-1 and a biologically active non-Angl protein.

66. The fusion protein of claim 65 wherein said non-ECM binding fragment of Ang-1 comprises SEQ ID NOs: 5, 6, 7, 8, 9, and/or 10.

67. A method of diagnosing an elevated probability of metastatic disease following tumor removal or elimination comprising detecting serum concentration of Ang-2 and/or c-Ang-1.

68. A method of diagnosing and evaluating cancer in an individual for its probability of being an aggressive malignant cancer comprising detecting the serum concentration of Ang-1.

69. A method of inhibiting Erk1/2 phosphorylation in a cell comprising administering a pharmaceutical composition comprising administering an effective amount of a phosphorylation inhibition fragment of Ang-1 to said cell.

70. The method of claim 69 wherein said phosphorylation inhibition fragment of Ang-1 comprises SEQ ID NO: 11 and/or SEQ ID NO: 12.

71. A method of inhibiting tumor angiogenesis in an animal comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an angiogenesis inhibiting fragment of Ang-1.

72. The method of claim 71, wherein said fragment is c-Ang-1.

73. The method of claim 71 wherein the composition is administered in conjunction with removal or elimination of a tumor.

74. The method of claim 71 wherein said animal is a mouse, rat, dog, cat, human, or primate.

75. A fusion protein comprising SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 and non-Ang-1 protein.

76. The fusion protein of claim 75 wherein said non-Ang-1 protein is a tag protein.

77. The fusion protein of claim 76 wherein said tag protein is a 6-Hisitidine Tag, an HA-tag, a GST-tag, a v5 epitope, or a myc tag.

78. A nucleic acid molecule encoding a fusion protein comprising SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 and non-Ang-1 protein.

79. The nucleic acid molecule of claim 78 wherein said non-Ang-1 protein is a tag protein.

80. The nucleic acid molecule of claim 79wherein said tag protein is a 6-Hisitidine Tag, an HA-tag, a GST-tag, a v5 epitope, or a myc tag.